## Second-strand cDNA Synthesis Primed by Oligonucleotides

Synthesis of the second strand of cDNA is usually primed by hairpin structures at the 3′ terminus of the first strand. An alternative procedure is to tail the first strand of cDNA directly with dT (Rougeon et al. 1975) or dC (Land et al. 1981). The second strand is then synthesized using an oligo(dA) or oligo(dG) primer, respectively, producing duplex cDNA flanked by duplex homopolymeric tracts at each end. The duplex DNA is then tailed with dC and inserted into a plasmid that has been cleaved with *Pst*I and tailed with dG.

The chief advantage of this procedure is that it eliminates the difficult step in which nuclease S1 is used to cleave the hairpin loop in double-stranded cDNA and thus facilitates the efficient cloning of full-length, double-stranded cDNA. One potential pitfall in this procedure is that even highly purified preparations of terminal transferase are contaminated with single-strand-specific nucleases. Presumably, this latter problem could be circumvented by tailing the first cDNA strand as a DNA · RNA hybrid.

## Plasmid-primed, First- and Second-strand cDNA Synthesis

Recently, a novel method for high-efficiency cloning of full-length, double-stranded cDNA was published by Okayama and Berg (1982). The steps in their protocol are as follows (see Fig. 7.3A,B,C):

1. A plasmid primer for cDNA synthesis is prepared by dT tailing with terminal transferase. A fragment containing one of the dT tails, the bacterial origin of replication, and the ampicillin-resistance gene is prepared by digestion with a second enzyme, followed by agarose gel electrophoresis and oligo(dA) cellulose chromatography (Fig. 7.3A).

2. An oligo(dG)-tailed linker DNA is prepared by dG tailing a *Pst*I DNA fragment with terminal transferase, followed by digestion with a second enzyme to separate the two ends. The desired end fragment is purified by agarose gel electrophoresis (Fig. 7.3B).

3. The dT-tailed vector-primer is annealed with poly(A) mRNA at a molar ratio of 1.5–3 (mRNA:vector-primer), and a first cDNA strand is synthesized with reverse transcriptase (Fig. 7.3C).

4. dC tails are added to the 3′ end of the cDNA copy while it is still hydrogen bonded to the mRNA template. The dC tail added at the other end of the vector is then removed by restriction endonuclease digestion.

5. The oligo(dG)-tailed cDNA · mRNA plasmid is annealed and ligated to the oligo(dG)-tailed linker DNA.

6. The mRNA strand is replaced by DNA using the combined activities of RNase H, which degrades the RNA strand in an RNA · DNA hybrid, *E. coli* DNA polymerase I, which carries out a nick-translation repair of the second cDNA strand, and DNA ligase, which covalently closes the circular DNA molecule.

Okayama and Berg find that full-length or nearly full length cDNA copies are preferentially converted to duplex cDNA, and an efficiency of approximately 100,000 transformants per microgram of starting mRNA is obtained. The preferential cloning of long cDNA transcripts is thought to be a consequence of the preferential utilization of full-length reverse transcription by terminal transferase. They speculate that shortened or truncated cDNA strands in the mRNA · DNA duplex are not efficiently recognized by the terminal transferase and are therefore selected against. Although the rabbit $\alpha$- and $\beta$-globin mRNA was used to establish this cDNA cloning procedure, Okayama and Berg indicate that other cDNA clones representing both rare and long (6500-nucleotide) mRNAs have been obtained with this procedure.

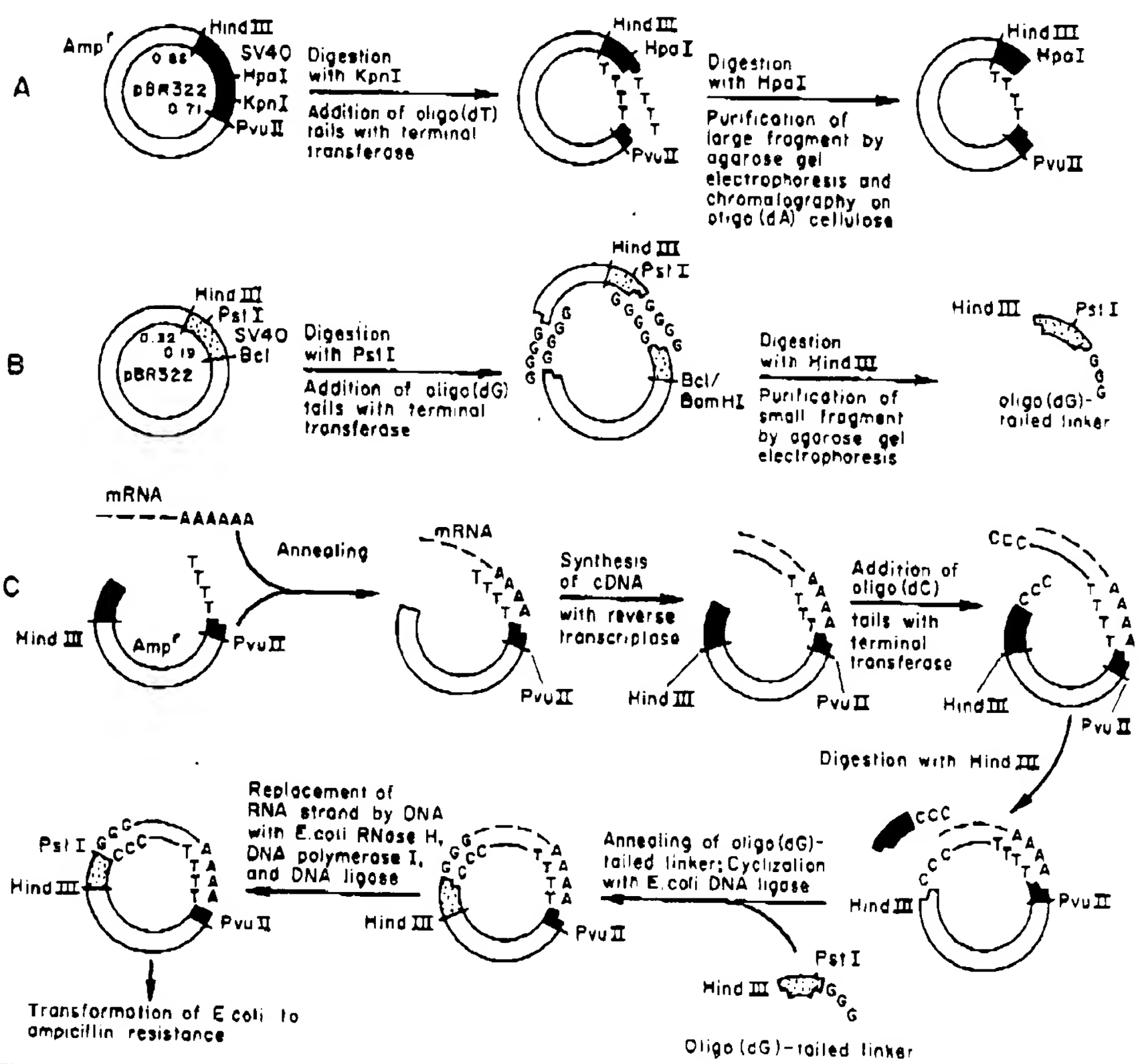


**Figure 7.3**

Preparation of (*A*) plasmid primer and (*B*) oligo(dG)-tailed linker DNA. (*C*) Steps in the construction of plasmid-cDNA recombinants. pBR322 DNA is represented by the open sections of each ring; SV40 DNA is indicated by the darkened or stippled segments. The numbers next to the restriction site designations are the corresponding SV40 DNA map coordinates.